METHOD FOR OPTICALLY MANIPULATING POLYMER FILAMENTS

FIELD OF THE INVENTION

The present invention relates to an apparatus for optically manipulating microscopic particles, and to a method for preparing nucleic acid fragments for examination in an extended form.

REFERENCES

Ashkin, A., et al. Optics Lett., 11(5):288 (1986).
Dunlap, D. D., et al., Nature, 342:204 (1989).
Humphries, Robertson, M., Nature, 306:733 (1983).
Maniatis, T., et al., *Molecular Cloning: A laboratory Manual*, Cold Spring Harbor Laboratory (1982).
Smith, S. B., et al., Science 243:204 (1989).
Wilcheck, M., et al., Anal Biochem 171:1 (1988).
Williams, C. C., et al., Nature, 344:317 (1990).

BACKGROUND OF THE INVENTION

Much of the current research effort in molecular genetics is aimed at localizing genes, determining relative gene positions along chromosomes or DNA filaments, and determining their nucleotide sequences. One major application of gene localization is in understanding and predicting certain genetic disease states. For example, traslocation of marker genes from one chromosomal location to another may play a role in the development of cancer (e.g., Robertson). Also a number of inheritable diseases have been identified by their genetic linkage to observed restriction fragment polymorphisms (e.g., Humphries), and considerable effort has been devoted to identifying the sites of the gene defects in particular chromosome regions associated with the polymorphisms.

Heretofore, gene and probe-site localization along a mammalian chromosome or DNA filament has been approached either by classical studies on gene linkage related to inheritance or by in situ hybridization techniques. In the gene linkage approach, the frequency of co-inheritance of one phenotypic trait, whose gene location is unknown, with a phenotypic trait whose gene location is known provides a measure of the distance (linkage) between the two genes. The classical approach is quite limited in man, where family inheritability patterns must be relied upon. Even in animals where controlled breeding is possible, genetic studies are unable to resolve distance of less than about 5 to 10 million basepairs.

Genomic DNA regions of unique sequence can be localized on a chromosome by in situ hybridization. Typically, this is done by hybridizing a radiolabeled probe with a single-strand filament which is also radiolabeled, but at a lower specific activity. The strand is then developed autoradiographically, and the probe is localized by counting the distribution of grains on the film. This method is quite slow, often requiring several weeks for film development and multiple samples in order to achieve statistically meaningful grain distribution patterns for probe localization. Even then, the method cannot resolve locations closer than about 5–10 million basepairs.

Although attempts to map the location of fluorescent-labeled probes on a DNA strand by fluorescence microscopy have been reported, this approach has been severely limited heretofore. A major limitation is the tendency of nucleic acid fragments to form supercoiled, essentially globular structures in solution, making it difficult or impossible to localize the probe or determine distance relationships among probes or between a probe and an end of the filament. The tendency of DNA to form tangles also frustrates direct sequencing using nanometer-scale probe microscopy, such as scanning-tunnelling microscopy.

According to one feature of the present invention, it is now possible to extend long nucleic acid filaments in solution, and to detect a single probe, such as a fluorescence-labeled DNA probe, with 100 base pair precision along a nucleic acid filament. The method for extending nucleic acid filaments in solution, in accordance with the invention, employs single-beam gradient force optical trapping to capture and move a microscopic particle attached to one end of a DNA filament. The experimental observation of single-beam optical trapping was first described by one of the inventors and his coauthors (Ashkin). Briefly, single-beam optical trapping employs a single, strongly focused beam in which the particle is trapped at a point near the focus of beam. The particle is held in the trap by the axial gradient force, which is proportional to the gradient of the light intensity and points in the direction of increased intensity.

The success of the single-beam optical trap depends on the ability to stabilize the particle at beam focus, and this in turn, is related to the intensity of the incident light beam at the point of focus and the strength of the axial gradient force. In general, the conditions necessary for single-beam optical trapping of particles can be achieved in a stationary-beam arrangement by directing a beam through a strongly convergent (high numerical aperture) objective lens (Ashkin).

In the method of the invention, where the optical beam is used to manipulate the position of a particle in a liquid film on a microscope stage, it is convenient to move the trapping beam relative to the stage, typically by moving the source beam to produce a selected movement in the trap. However, if the source beam is simply moved with respect to the surface of the optical trap (objective) lens, by a mirror or lens steering the trapping beam, the intensity of light (and thus the trapping force) at the trap will vary with position, making it difficult to maintain the beam in a trapped condition as the beam is manipulated.

SUMMARY OF THE INVENTION

It is one general object of the invention to provide a single-beam optical trapping apparatus which produces an optical beam whose trapping force is substantially independent of position within a view field.

Another general object of the invention is to provide an apparatus and method for preparing and examining nucleic acid filaments in an extended form.

In one aspect, the invention includes apparatus for manipulating a particle in the size range of about 10 nm to 10 μm by single-beam gradient optical trapping, and typically between about 0.1 and 1 μm. The apparatus includes a chamber which supports a film of fluid in which the particle can be immersed and through which the particle can be moved. The optical trap is produced by directing a collimated beam of coherent light through a high-numerical aperture objective lens, with the beam substantially filling the lens. The collimating beam is produced by directing a divergent, coherent beam from a movable light source through a collimating lens which is positioned to (a) shift the angle by which